

**A DISSERTATION ON THE ISOLATION, CULTURE,
SUB-CULTURE AND SPECIES IDENTIFICATION
OF CANDIDA FROM THE MUCOSAL LESIONS OF
IMMUNOCOMPROMISED INDIVIDUALS**

Dissertation Submitted to

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DR.M.G.R. MEDICAL UNIVERSITY**

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M.D. (Dermatology, Venereology and Leprology)

BRANCH - XII A



**MADRAS MEDICAL COLLEGE
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DECLARATION

I, **Dr.K.RAJKUMAR**, solemnly declare that dissertation titled, **“A DISSERTATION ON THE ISOLATION, CULTURE, SUB-CULTURE AND SPECIES IDENTIFICATION OF CANDIDA FROM THE MUCOSAL LESIONS OF IMMUNOCOMPROMISED INDIVIDUALS”** is a bonafide work done by me at Madras medical college during 2006-2008 under the guidance and supervision of **Prof. Dr. B. PARVEEN, M.D., D.D.**, Professor and Head, Department of Dermatology, Madras Medical College, Chennai - 600 003.

The dissertation is submitted to The Tamilnadu, Dr.M.G.R. Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree in Dermatology, Venereology and Leprology (BRANCH - XII A)**.

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INTRODUCTION

Infections caused by the yeasts of the genus *Candida* are common in clinical practice. They may involve the mucous membranes and / or skin or spread internally to produce systemic infections.

Superficial infections of the mucous membranes and skin are numerically most important but more serious involvement of the internal organs as in septicemia, endocarditis, meningitis, can also occur.

Candida albicans is an oval yeast 2-6 x 3-9 μm in size, which can produce budding cells, pseudohyphae and true hyphae. The ability to simultaneously display several morphological forms is known as polymorphism. Although hyphae are likely to be produced during the process of tissue invasion, yeasts without hyphae may also occur in invasive disease, particularly in infections caused by non-*albicans* *Candida* species.

Candida species are normal commensals of the human beings. They are commonly found throughout the gastrointestinal tract, female genital tract and anterior urethra. They cause opportunistic infections in man. They have many functions inside our digestive tract and one of them is to recognize and destroy harmful bacteria.

There are more than 200 species (1) of candida but the following are considered to be important pathogens for humans : *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida guilliermondii*, *Candida glabrata* (now classified as *Torulopsis glabrata*) *Candida parapsilosis*. *Candida albicans* is the most commonly isolated species and accounts for 90% of cases of invasive Candidiasis. Trichosporon species which resembles *Candida krusei* have recently been isolated from clinical specimens. Candida species are the fifth most common primary blood stream invaders and the seventh most common pathogen to cause nosocomial infections (1).

Candida species grow easily on Sabouraud's Dextrose Agar at 37°C at room temperature within 24-48 hrs. The colonies are white or cream coloured, smooth with a yeasty odour that are naturally present as a part of the normal commensal in the digestive system. A problem can occur when there is an abnormal overgrowth of the yeast, that is normally controlled by the "Beneficial bacteria" in the intestines. When factors such as antibiotics, steroids, and refined sugar are used in excess, since bacteria and specific nutrients in the gut are destroyed imbalanced yeast can overgrow. The immune system and therefore our body's resistance is then lowered and the yeasts begin to invade and colonise the tissues. These yeasts release toxic chemicals into the blood that causes varying symptoms. These toxins attack the immune system, permitting the fungi to continue their tissue invasion and to cause more serious symptoms.

REVIEW OF LITERATURE

The history of Candidiasis dates back to the 4th century B.C. when Hippocrates, described oral aphthae (thrush) in two patients with severe underlying disease. Rosen Von Rosenstein in 1771 and Underwood in 1784 recognized thrush as a condition in infants and described oral and gastrointestinal thrush. In 1835, Veron first described a case of esophageal candidiasis and postulated that new borns acquire the disease during the passage through the vagina. The initial discovery of the thrush organism was made in 1839 by Langenbeck, who observed a fungus in scrapings of buccal thrush from a patient with typhus. In 1842, Gruby, described the thrush fungus before the academy of sciences at Paris as "Levrai muguet des enfants" and placed it in the genus *Sporotrichum*.

Two years after Gruby's report, Bennett illustrated the microscopic characteristics of a fungus that appeared to be *Candida albicans* in the sputum and lung of a patient who had tuberculous pneumothorax. In 1849, wilkinson first described vaginal Candidiasis and its mycotic origin, the dimorphic nature of thrush fungi was noticed by Grawitz in 1877. He described the budding yeast form, the mycelial form, and chlamydospores, although he did not name these structures. Ten years later, Audrey proved that the diverse morphologic forms are produced by the same strain, depending on environmental conditions. In 1890, Schmorl reported a case of disseminated Candidiasis involving

many organs. During the following 50 years, reports followed on the various different forms of Candidiasis including Onychomycosis, Chronic mucocutaneous disease, Cystitis, Endocarditis, Osteomyelitis and Endophthalmitis. In 1890, Zopf named the thrush fungus *Monilia albicans*, from which Moniliasis, the early name of Candidiasis, originated. As defined by Saccardo in 1886, the genus "monilia" included certain filamentous fungi isolated from rotting fruits and leaves. In 1923, Berkhout noted that the medical "Monilia" species differed physiologically and morphologically from the fruit-rotting Monilia. He established the genus *Candida* to accommodate "Monilia" and defined it to include anascosporogenous yeast species that develop pseudohyphae. During the first two decades of this century, Castellani made extensive studies on mycoses caused by yeasts. His report of "Tea-Taster's cough" in 1912 has been considered to be an early account of broncho-pulmonary candidiasis. He suggested that yeast species other than *Monilia albicans* might be involved in candidiasis and made the first description of the species currently known *C.guilliermondii*, *C.krusei*, *C.kefyr* (*C.pseudotropicalis*) & *C.tropicalis* (2).

Fungal infection in human being ranges from those involving only the most superficial areas of the body to those in which subcutaneous tissue is attacked, to those deep and systemic infections that often involved most of the major organs of the body. The last decade has seen a marked increase in systemic mycoses, an increase that has been matched by the recent introduction of new systemic antifungal agents.

CHARACTERIZATION OF CANDIDA SPECIES

Candida Species are budding yeast like fungus and they produce pseudohyphae also.

Candida albicans

On Sabouraud's Dextrose Agar (SDA), the colonies are cream coloured, pasty and smooth. In cornmeal agar at 25°C for 72 hrs, pseudohyphae are seen in clusters. Large thick-walled, terminal, chlamydospores are the characteristic of this species. They form germ tube on incubation with human serum. They do not produce a pellicle on Sabouraud's dextrose broth.

Candida tropicalis

On Sabouraud's dextrose agar, the colonies are cream-coloured, smooth, wrinkled with mycelial fringe. On corn-meal agar, it forms blastospores singly or in small groups. It produces narrow surface film with bubbles on Sabouraud's dextrose broth.

Candida krusei

On Sabouraud's dextrose agar, the colonies are dull, dry, wrinkled and smooth. The elongated cells have tree like arrangement or crossed matchsticks appearance. It produces wide pellicles on the sides of tube on Sabouraud's dextrose broth. Urease test is a positive reaction and is indicated by a purple-pink or red colour of Christensen's urease agar medium.

Candida glabrata

The colonies on Sabouraud's Dextrose Agar are cream coloured, smooth, soft and they produce no pseudohyphae. These are small round or ovoid yeast cells. They are also called *Torulopsis glabrata*. They do not produce pellicles on Sabouraud's dextrose broth.

Candida parapsilosis

On Sabouraud's Dextrose Agar the colonies are cream coloured, shiny, smooth. It produces blastospores singly or in small clusters that are seen along the pseudomycelium. They do not produce pellicles on Sabouraud's dextrose broth.

Candida guilliermondii

On Sabouraud's Dextrose Agar, the colonies are thin flat, glossy, cream to pink, smooth or dull, wrinkled. Variable development of pseudomycelium that are fine and short with small cells, some times bearing ramified chains of small ovoid blastospores.

Trichosporon species

On Sabouraud's Dextrose Agar, the colonies are creamy coloured moist, soft, wrinkled. They produce arthrospores, budding yeast like cells, and pseudohyphae are also seen. They produce wide pellicles on Sabouraud's dextrose broth.

Rhodotorula species

On Sabouraud's dextrose agar, the colonies are pink to red soft, smooth. On cornmeal - Tween 80 agar at 25°C for 72 hrs budding cells that are round or oval and pseudohyphae are seen. Urease test is positive and the reaction is indicated by a purple-pink or red colour of the medium. They do not produce pellicles on Sabouraud's dextrose broth.

CANDIDA ECOLOGY AND THE COMMENSAL OR PARASITE ROLE (3)**Gastrointestinal tract carriage**

Many species of animals and birds carry yeasts, often species of candida in their gut as part of the normal commensal flora and the human is no exception. Candida albicans is a frequent but not invariable inhabitant of the gastrointestinal tract. Colonization occurs during birth directly from the birth canal, at sometime during infancy or perhaps later in life.

In his review Odds (4) concludes that fewer than 26% normal subjects carry yeasts in the mouth and that the figure for C.albicans carriage is about 18%. If specialized techniques or repeated sampling are used, the proportion of healthy adults carrying candida in the gastrointestinal tract may be demonstrably higher.

Vaginal carriage (5)

The healthy vagina may be colonized by yeasts - most commonly *C.albicans*, sometimes *C.glabrata*, but only in a minority of women(6). The percentage of vaginal carriers differs widely in different surveys, but a figure of 12.7% for *C.albicans* is probably accurate. Higher rates are found in hospital patients, even without vaginal disease [4].

Cutaneous carriage

Neither *C.albicans* nor any other species of *Candida* is a permanent member of the normal flora of the skin. At the same time, it is clear from numerous surveys that skin adjacent to the body orifices and the skin of the fingers, which are in frequent contact with the mouth, often yield *C.albicans* and sometimes other species, particularly *C.parapsilosis* and *C.guilliermondii*(7). In moist intertriginous sites, candida may be a persistent colonizer in a few individuals. Age and climate are important in this connection. Samples from the very young and the very old are more likely to yield candida.

Carriage in other sites

The bronchial tree is not normally colonized by *Candida* and whenever the organism is isolated from sputum specimens, at least in low amounts, it can be assumed that it has come from the mouth or oropharynx. *Candida albicans* can occasionally be cultured from the

environment, usually in situations where there are heavily infected subjects(4). Normally however, candida is not part of the air-borne microflora. Except for the neonatal and conjugal infections, most of the cases of candidiasis probably result from infection of the host by his or her own commensal yeasts. *Candida albicans* may also demonstrate an unusual phenomenon known as “phenotypic switching”, whereby a strain may change morphology or another phenotypic character such as drug sensitivity in response to a change in growth conditions. Such changes are reversible and not associated with genetic variation (8).

PATHOGENESIS

Fungal virulence

The most common pathogen in skin disease is *C.albicans*, although increasingly other species are isolated in vaginal infections and from AIDS patients.

Enzymes and Toxins

Factors such as the production of an acid proteinase by certain strains of *C.albicans* are also known to affect pathogenicity. Proteinase - negative strains are known to be less virulent (9). Laboratory - generated gene defective strains have not been shown to be less virulent.

Yeast-mycelial shift (10)

In oral and cutaneous candidiasis, scrapings examined microscopically usually show candida in both budding and mycelial

forms. In histopathology of invasive candidiasis hyphae are usually present. This suggests that the production of hyphae may contribute to fungal virulence.

Adherence

The ability of yeast forms to adhere to the underlying epithelium is also an important prerequisite for tissue invasion [11-13]. Adherence to epithelial surface is mediated through a number of receptor interactions. Candida adhesins are either based on cell wall mannan or protein components. Among the latter is a candida surface C3d - binding protein [6]. It has also been shown that proteinase, production is necessary for adherence.

HOST FACTORS

Host factors involved in mucocutaneous candidiasis are numerous. Any form of local tissue damage may be important in the pathogenesis of candidiasis. Experimental removal of the stratum corneum facilitates the establishment of cutaneous candidiasis and with a given inoculum increases the severity of the response.

FACTORS PREDISPOSING TO CANDIDA INFECTION

- **Mechanical factors** : Trauma, local occlusion, moisture, maceration dentures, occlusive garments and obesity.
- **Nutritional factors** : Avitaminosis, iron deficiency, generalised malnutrition.
- **Physiologic alteration** : Extremes of age, pregnancy and menstruation.
- **Systemic illness** : Down's syndrome, Acrodermatitis enteropathica, Diabetes mellitus and certain other endocrinopathies viz., Cushing's syndrome, hypoadrenalism, hypothyroidism, hypoparathyroidism.
- **Intrinsic immunodeficiency**
States : DiGeorge's syndrome, Nezelof syndrome, severe combined immunodeficiency syndrome, myeloperoxidase deficiency, Chediak - Higashi syndrome, Hyperimmuno globulinemia E syndrome.

Chronic Granulomatous Disease - AIDS

Iatrogenic causes

Barrier weakening factors, indwelling catheters, I.V. drug abusers, X-ray irradiation.

Drugs

Corticosteroids and other immunosuppressive agents, antibiotics, oral-contraceptives, especially estrogen dominant, colchicine, phenylbutazone.

Local tissue damage

The pathogenesis of candidiasis is favoured by any form of local tissue damage. Use of artificial dentures, favours oral candidiasis. Maceration and moisture favour the growth of candida on the skin. Studies have shown an increased incidence of candidal paronychia in psoriatic patients, also increased levels of candida carriage in psoriatic or eczematous skin.

Role of iron

Iron deficiency has been found to be associated with chronic mucocutaneous candidiasis. However in vitro studies have shown that unsaturated transferrin acts as an inhibitor of *Candida albicans*. This inhibitory action is mediated via direct binding of the molecule to the yeast cell. Iron reverses this effect.

Serum Factors

A serum factor has been described that reduces the number of colony forming units by clumping but in practice it may increase the susceptibility to infection. Persistent candidiasis sometimes leads to the production of a serum factor that causes T cell inhibition.

Endocrine Factors

Like diabetes mellitus, Cushing's syndrome also increases the susceptibility to candidiasis by suppressing the immune system. Apart from these, other endocrinopathies associated with candidiasis are Addison's disease, hypoparathyroidism and hypothyroidism that occur in candidal endocrinopathy syndrome.

Immunological factors

Cell-mediated immunity (CMI) plays a major role though humoral immunity may also have some role. Phagocytosis by polymorphs and macrophages is also important (14, 15). T-cell function is depressed in chronic mucocutaneous candidiasis, which is associated with the absence of specific anti-candida secretory IgA antibody (16). Systemic steroids increase the susceptibility to candidiasis by suppressing the T-cell function though topical steroids reduce the inflammatory reaction produced by dead and disintegrated candida cells

or living organism. Decreased CMI in the elderly and debilitated persons, in patients with Malignancies and with AIDS makes them more prone to mucosal or cutaneous candidiasis, but not to systemic infections, whereas patients with defective neutrophil / macrophage function are susceptible to systemic candidiasis.

Patients with defective T-lymphocyte function, such as those with AIDS, appear to be particularly susceptible to mucosal or cutaneous candidiasis, but not systemic infections (17). Congenitally T-cell deficient - mice (nu / nu) do not show reproducible increased susceptibility to systemic infection by candida. In fact some investigators have found heightened resistance, suggesting that T-lymphocyte activity alone does not account for resistance to systemic invasion. In contrast, in patients with chronic mucocutaneous candidiasis, the most consistent abnormalities have been those of T-lymphocyte function, particularly cytokine expression (18), even though some of these are now thought to be secondary to immunoregulation induced by the infection.

Patients with defective neutrophil or macrophage function are susceptible to both superficial and systemic candidiasis. The activity of neutrophils and macrophages in phagocytosis and killing of candida in-vitro has been demonstrated. In addition some cytokines such as

interferon- γ appear to interact with these cells to enhance killing of the organism. It appears that there is therefore substantial interplay between different immune mechanisms in defence against candidiasis.

CANDIDIASIS AND AIDS

In the untreated HIV - Positive population, oral candida carriage rates are generally high and this has been confirmed by the finding that carriage rates are, for instance, higher in HIV positive homosexual males than in a control group of HIV-negative homosexual men (1). Colonization rates were higher in intravenous drug abusers, Center for Disease Control (CDC) group IV and in those with lymphopenia. In addition, patients with CD₄ cell depletion and those with elevated β_2 microglobulin levels were more likely to be carriers (19).

Oral thrush does appear to reflect viral load (20). Both hairy leukoplakia and oral candidiasis are markers for increased rate of progression to AIDS. The presence of oral candidiasis may also be a marker of survival in some patients. For instance, HIV positive patients with oral candidiasis but who have no other features of AIDS have a poorer survival rate than those without (21). An important factor implicated as a possible predisposing cause of oral candida infection in AIDS patients is the salivary flow rate.

CLINICAL SYNDROMES OF CANDIDOSIS

Oral Candidosis (22)

Acute pseudomembranous candidosis

(synonym - oral thrush) (23,24).

Clinically characterised by a sharply defined patch of creamy, crumbly, curd-like white pseudomembrane, which when removed, leaves an underlying erythematous base. This membrane consists of desquamated epithelial cells, fibrin, leukocytes and fungal mycelium that attaches it to the inflamed epithelium. There may be one or many patches. The buccal epithelium on the cheeks, the gums or the palate may be affected; Tongue is also involved in the immunocompromised patients. It may be present in the neutropenic patient or those with AIDS (25-27).

In both cases, the clinical changes are often erosive with severe symptoms resulting in inadequate food intake because of pain. Extension of erosions to the buccal mucosa, tongue and esophagus is common.

Acute erythematous candidosis (Acute atrophic oral candidiasis) (28)

In this condition there is marked soreness and denuded atrophic erythematous mucous membranes, particularly on the dorsum of the

tongue. It may follow pseudomembranous candidosis when traces of the residual membrane will often be found. It is especially associated with antibacterial antibiotic therapy and can also occur in HIV positive subjects.

Chronic Pseudomembranous Candidosis

This doesn't differ clinically from the acute pseudomembranous variety but as the name suggests lesions are very prominent and persistent. It occurs principally in immunocompromised patients.

Chronic Erythematous Candidosis (Chronic atrophic candidiasis) (29)

Denture sore, denture stomatitis.

Some soreness in the epithelium in the denture - bearing area is said to affect nearly one quarter of all denture wearers. A similar problem may also occur in children wearing orthodontic appliances.

Chronic mechanical irritation and bacterial colonisation have a role in the pathogenesis of this condition.

Chronic plaque like candidosis (Chronic hyperplastic candidiasis) (30)

Very persistent, firm, irregular white plaques occur in the mouth, commonly on the cheek or the tongue. Around the hyperplastic area,

there may be a margin of erythema. Unlike the pseudomembrane of oral thrush, this plaque cannot be easily removed. Serious predisposing factors are not present. This type has to be differentiated from other types of leukoplakia. Although the affected areas may undergo malignant (31, 32) change it may eventually clear with prolonged anti-candida therapy.

Chronic nodular candidosis

This is a rare form, where the clinical appearance that usually affects the tongue is of a cobbled appearance. It is most often seen in certain patients with chronic mucocutaneous candidosis.

Angular cheilitis (Angular Stomatitis; Perleche) [33]

Soreness at the angles of the mouth extending outwards in the folds of the facial skin is a well known syndrome. Nutritional status and mechanical factors (eg. the depth of the fold), the presence of moisture from persistent salivation or licking the lips may also be important. The yeasts involved clearly come from the mouth, and the association with denture stomatitis is important. Although the condition may present acutely, it is common to find a long history of soreness and cracking at the angles of the mouth and a fluctuating course is typical.

Median rhomboid glossitis (34)

This condition characterised by a more or less, diamond shaped area on the dorsum of the tongue with loss of papillae, occurs as an acquired condition. It has been regarded in the past as a developmental abnormality, but current opinion suggests that it is simply a variant of chronic plaque - like candidosis.

Candidosis, steroids and the mouth

Apart from systemic steroid therapy, local applications of steroids in the form of steroid creams, mouth washes and lozenges for the treatment of aphthosis or lichen planus of the mouth may predispose to candidosis, sometimes occurring as a secondary invasion of the primary pathology. Similarly, steroid aerosols, for asthma must be considered as at least a potential cause of diminished local immunity in this area.

Candidosis of the skin and genital mucous membranes

Most cases of cutaneous candidosis occur in the skin folds or where occlusion from clothing or medical dressings produces abnormally moist conditions. Areas close to the body orifices and the fingers, which are frequently contaminated with saliva are also at risk.

Candida intertrigo (Flexural candidosis)

In obese subjects, any skin fold may be affected. Signs are typically erythema and a little moist exudation starting deep in the fold. As the condition develops, it spreads beyond the area of contact, usually developing the typical features of candidosis with a fringed irregular edge and subcorneal pustules rupturing to give tiny erosions and further peeling of the stratum corneum. Satellite lesions, pustular or papular are classical. Soreness and itching on occasions may be intense. In case of hands some abnormality including wide fat fingers, appears to predispose to infection. In this particular syndrome, known as Erosio interdigitalis blastomycetica or interdigital candidosis, candida and gram negative bacteria are often co-pathogens (35). Apart from skin folds, macerated skin under rings and dressings may become infected with candida.

Differential diagnosis of intertriginous candidosis includes, tinea infection, seborrhoeic dermatitis, bacterial intertrigo, flexural psoriasis, Hailey-Hailey disease and flexural Darier's disease.

Vulvovaginitis (Vulvo-vaginal thrush) (36, 37)

Common condition, presents with itching and soreness, and with a thick creamy white discharge. Most women with vaginal candidosis have no evidence of underlying disease.

More common in pregnancy. In the non-pregnant it is said to be more prevalent in the premenstrual phase, but a fluctuating course not clearly related to the menstrual cycle is frequent. Although largely confined to sexually active subjects it has been described in childhood, sexually inexperienced and elderly people. Typically there is dusky red erythema of the vaginal mucosa and the vulval skin with curdy white flakes of discharge but on occasions the only sign is erythema. Candidial vulvo vaginitis may recur and in some it appears to be a chronic condition (38). Management of the recurrent or chronic case is difficult; the condition causes considerable distress.

Candidial balanitis (39)

Skin of the glans penis especially in the uncircumcised, may sometimes be colonized by candida asymptotically (4).

When candidial balanitis develops, it is usual to find either abundant vaginal candida carriage or frank vulvo-vaginitis in the sexual partner although this is variable. In the mildest cases transient tiny papules or pustules develop on the glans penis, a few hours after intercourse, and rupture leaving a peeling edge. This mild form is usually associated with a little soreness and irritation. In the male, failure to find the organism does not exclude the diagnosis if swabs or scrapings were not taken during the acute phase. It is wise to consider diabetes in cases of genital yeast infections.

Perianal and scrotal candidosis

May occur with or independently of genital involvement. Although usually starting around the anal margin with non-specific erythema, soreness and irritation subsequently spreading along the natal cleft is common with classical features developing as it extends. Candidosis must be included in the differential diagnosis of unexplained erythema of the scrotal skin. Secondary infection of flexural psoriasis with candida may have to be considered.

Napkin candidosis (Diaper candidiasis)

Candida albicans is commonly isolated from the moist skin of the buttocks and genitalia of the infant but is more prevalent where the skin is affected by napkin rash (40). In some instances, the classical subcorneal pustules, a fringed irregular border and satellite lesions are found. If the bacterial flora has been suppressed by a topical antibiotic this will also favour the yeast.

Nodular or granulomatous candidosis of the napkin area (granuloma gluteale infantum) (41, 42)

Clinical picture is that of a napkin eruption over the buttocks, genitalia, upper thighs and pubis, within which develop nodules,

sometimes as large as 2 cm across, bluish or brownish in colour, reminiscent of Kaposi's Sarcoma. The primary napkin dermatitis may clear leaving only the nodules.

Candidial paronychia

Candidial species can be isolated from the majority of the cases of chronic paronychia (43, 44). The yeast is thought to have an etiological role in this condition, but bacteria and irritant or allergic contact dermatitis also play a part, although the contribution of each varies from patient to patient. Condition is common among those whose hands are frequently immersed in water, but in chefs and pastry cooks the presence of organic debris such as flour and other carbohydrates may equally be important.

Clinical features

Typically several fingers are chronically infected, but one or all may be involved. The nail fold is red and swollen and there is loss of the cuticle, and detachment of the nail fold from the dorsal surface of the nail plate, leading to pocketing.

Onychomycosis resulting from candida

Erosion of the Distal and lateral nail plate of the fingernails not usually progressing to total nail dystrophy has been associated with *C-albicans* invasion of the nail (DLSO) (45). Two important predisposing conditions are Raynaud's Phenomenon or disease and Cushing's syndrome. Rarely candida may invade the nail plate in the neonatal period, sometimes causing an isolated nail dystrophy with evidence of penetration of the superior aspect of the nail plate (SWO).

Deep - seated candidiasis

The term 'deep-seated candidiasis' (Odds 1988, Bodey 1993, Edwards 1995) refers to infection of visceral organs and possibly to multiple organ or disseminated disease. Clinical entities include, candidiasis of the gastrointestinal tract, respiratory system, central nervous system, renal and urinary tract, cardiovascular system' hepatosplenic candidiasis, haematogenous disseminated disease and ocular infections.

CANDIDIASIS OF THE GASTROINTESTINAL TRACT

Oesophagitis

This syndrome (Musial, Cockerill and Roberts, 1988) includes odynophagia and chest pains. Additional symptoms may include nausea and / or vomiting. White patches, which resemble those of oral candidiasis, can be noted by endoscopy on the esophageal mucosa. Oesophagitis may be associated with the presence of oral candidiasis, but it may also present as a separate clinical entity. The frequency of this syndrome has increased since the emergence of AIDS. It is estimated that 10-30% of AIDS patients with oral candidiasis may also have candidial esophagitis. This infection is also seen in cancer patients after anti-cancer therapy.

GIT candidiasis

Candida species primarily *C.albicans*, *C.glabrata* and *C.tropicalis* colonise the gastrointestinal tract in a significant proportion of normal individuals (Stone et al., 1973, Bolivar and Bodey 1985, Odds 1988) as judged by fecal fungal isolation. An increase in percentage of colonized individuals is noted among the hospitalized patients. (Kusne et al., 1994).

Candidal gastrointestinal colonization and infection are believed to play a role in the pathogenesis of disseminated candidiasis. The gastrointestinal tract can serve as a reservoir for the fungus, from where it can spread, particularly if there is a breach in the mucosal lining.

Candidiasis of the liver, spleen and other organs

Hepatosplenic candidiasis is seen primarily in individuals with leukemia, with hepatosplenic involvement becoming apparent during recovery from the neutropenic state. This clinical entity is difficult to diagnose and manage. Diagnosis can be assisted by Computed Tomography (CT) demonstrating lesions in the liver and spleen.

Candidiasis of the respiratory system

Respiratory candidiasis involving the lungs or bronchial system appears predominantly in patients with underlying primary diseases. (Masur, Rosen and Armstrong 1977, Gueteau et al., 1991). Bronchopneumonia can originate from haematogenous spread of the fungus as part of a disseminated infection or from introduction of the pathogen into the lung. Diagnosis is difficult because candida species are found in the sputa of individuals without candidiasis, so that it is pertinent to demonstrate the presence of the fungus in bronchopulmonary tissues.

Candidiasis of the Cardiovascular system

Candida species can cause clinical manifestations in various organs of the cardiovascular system. (Musial, Cockerill and Roberts, 1988; Kwon-Chung and Benneh, 1992, Edwards 1995). Pericardium,

myocardium and the endocardium can be involved with endocarditis being the best known clinical entity.

Endocarditis (Hallum and Williams 1993, Wilson et al., 1993.a) is seen primarily in intravenous drug users and in individuals with impaired or prosthetic heart valves. It has also been described in patients after various cardiac surgery procedures or as sequelae of anti-cancer therapy. As a result of the increase in drug addiction and frequency of cardiac surgery an increase in the prevalence of candida endocarditis was noted. Endocarditis can be caused by *C.albicans* as well as *C.parapsilosis* (Cancelas et al 1994) and *C.tropicalis*.

RENAL AND URINARY TRACT CANDIDIASIS

Lower urinary tract infection

Candidal lower urinary tract infection (UTI) is quite frequently seen in association with indwelling catheters. It is seen more frequently in women. The infection is also found in diabetic patients (Musial, Cockerill and Roberts, 1988, Gentry and Price, 1993). An interesting clinical feature is the possible formation of fungal masses (Fungus balls). (Scerpella and Alhalel, 1994) which may cause obstruction and impair normal urine flow.

Renal Infection

Renal candidiasis can theoretically, originate from haematogenous dissemination of candida spp. or as an ascending UTI. Renal candidiasis is characterised by microabscess formation; this is primarily evident in the cortex of the kidneys. The kidneys are a target organ for the fungus in infections induced by intraperitoneal or intravenous inoculations.

Central Nervous System Candidiasis

Involvement of the Central Nervous System (CNS) by Candida species is uncommon and limited predominantly to *C.albicans*. The most susceptible individuals are AIDS patients and pre-term infants. (Fakes, 1984, Baley et al., 1986, Hughes, Lepow and Hill 1993, Edwards 1995). The central nervous system is generally seen as part of disseminated candidiasis involving, primarily the meninges, although the abscess formation in brain tissue has also been reported. The cells of Candida species may be detected microscopically in direct smears from Cerebrospinal Fluid (CSF). In addition abnormal protein and / or sugar values may be found in CSF.

Disseminated candidiasis and candidemia

Can be defined as a multi-organ infection including possible candidemia, although blood cultures do not always yield the fungus.

Disseminated candidiasis caused by *C.albicans* and other species is associated with debilitation. Candidemia may present with the non-specific symptoms of a septic state, including fever, presence of pustules and muscle tenderness.

Ocular candidal infections

Candida species can affect both the outer and inner eye. Infection may originate from haematogenous dissemination or from direct fungal introduction. Eye involvement is caused by the following species viz., *C.albicans*, *C.glabrata*, *C.krusei* and *C.parapsilosis*. Endophthalmitis is generally a result of fungal haematogenous spread, although it may also result from exogenous sources, can cause total loss of vision and is characterised by the presence of typical white cotton-like lesions which as indicated are an important diagnostic criteria for disseminated candidiasis.

CHRONIC MUCOCUTANEOUS CANDIDIASIS

Persistent candida infection of the mouth, the skin and the nails refractory to conventional topical therapy is a distinct syndrome occurring as a more or less, isolated feature. Sometimes it is associated with a variety of other infections, both cutaneous and systemic (46, 47). In the latter case, it may represent a manifestation of a primary defect in immune function, for example severe combined deficiency. (Swiss-type agammaglobulinemia).

Clinical Features (46)

But for minor variations, the syndrome consists of the following features, usually starting in infancy or early childhood.

1. Persistent oral thrush responding only partially to conventional therapy or relapsing promptly after apparently successful treatment. Chronic hypertrophic changes may follow.

2. Cutaneous Candidosis : Often intertriginous skin is involved, but also the face and the hands and sometimes it is widespread over the trunk and limbs. In long standing lesions, the cutaneous changes are often atypical suggesting ringworm. Dermatophytosis in such patients may present in a similar manner.

3. Paronychia is commonly a feature, often with serious nail plate invasion and total dystrophic onychomycosis (48). The important findings are nail invasion at an early age often proceeding to complete nail involvement.

Patients with this syndrome comprise a heterogenous group. It is probably best to exclude from the syndrome of chronic mucocutaneous candidosis, those patients who present with a well-documented underlying immune defect, such as severe combined immunodeficiency or agammaglobulinemia, where severe candidosis may form a minor part of the secondary infectious complications. In these patients other

serious infections such as recurrent pneumonia or aspergillosis may occur.

Within the childhood onset group, there are a number of different variants that show features in common. Such features should not be taken as inflexible markers of a particular type, as there is probably considerable overlap in clinical expression between the different groups.

The different types are as follows :

1. Autosomal recessive CMC [49]

Usually starts in the first decade with persistent oral and nail plate infections. They do not develop endocrine defects and also tend to improve with increasing age.

2. Autosomal dominant CMC [50]

Usually more severely affected, than those with the recessive variety and other infections such as dermatophytosis may be particularly troublesome.

3. Idiopathic CMC [51]

This form was named diffuse CMC by Higgs and Wells in children who had no evidence of genetic predisposition. Their candidosis is also very severe with esophageal involvement and appearance of 'granulomas'.

The term candida granuloma was originally used to describe these severely affected patients, who may produce sheets of hyperkeratosis caused by candida infection on the skin and scalp.

4. CMC associated with endocrinopathy

Majority of these patients appear to have the Familial polyendocrinopathy syndrome [52,53]. Usually seen in early childhood, and occasionally the onset of the infection may predate the appearance of endocrine disease by as much as 10 years. Main cluster of endocrine abnormalities are hypoparathyroidism with hypoadrenocorticalism. Other autoimmune abnormalities like pernicious anemia, vitiligo (54) and ovarian failure can occur. Condition is also inherited as an autosomal recessive condition. Another group of CMC patients with associated hypothyroidism has also been noted.

5. Late onset CMC (55, 56)

Occasionally adult patients are found to have the syndrome of CMC. Cases have been associated with a thymoma, but the occasional sporadic infection in a patient with no detectable abnormality may be recognised.

IMMUNOLOGICAL CLASSIFICATION

It is still not possible to correlate precisely defects of immune function, with different clinical variants of the CMC syndrome and indeed with current investigative techniques, a substantial minority of cases have no demonstrable defect of immune function at all. Certain antigenic components of *C.albicans*, such as mannan as well as some glycoproteins are immunomodulatory [57]. Reversal of immune defects, such as absent delayed - type hypersensitivity to candida antigens, has been with successful clearance of candidosis in CMC patients. It is therefore possible that some of the immunological changes may be secondary to the infection itself.

DIAGNOSIS

The diagnosis of CMC normally requires, the elapse of time and repeated failure to respond to conservative treatment. Confusion may occur with persistent ringworm infections. A family history is of obvious importance and special note of full endocrine investigation is indicated.

ANTIGENICITY, IMMUNE RESPONSE AND IMMUNITY

In defining the antigenicity of candida species, primarily that of candida albicans most of the studies, particularly the earlier investigations concentrated on the cell wall.

The mannan - protein complex, particularly the polysaccharide moiety of the cell wall, is the major antigenic component. Antigens both heat labile and heat stable are present.

IMMUNE RESPONSE

Exposure to candida species, stimulates, both humoral and cell mediated immune responses. The antibodies represent the different immunoglobulin types IgG, IgA, IgM or IgE. IgG and IgM are generally found in sera of patients with deep seated candidiasis except in highly immunosuppressed patients, who are unable to mount an immune response. IgG and IgM antibodies can also be found in the mucocutaneous forms of candidiasis, so that they cannot be used effectively, for differentiating between mucocutaneous candidiasis and deep seated candidiasis.

LABORATORY DIAGNOSIS

Direct Examination

Clinical specimens from diseased skin or nails can be collected by scraping the affected area or by the use of swabs.

Preferred method for direct examination of clinical specimens from cutaneous and nail candidiasis is the wet mount technique. Specimens have to be treated in most cases, with a keratinolytic

substance, generally 10-30% KOH, which facilitates microscopic examination of the specimen.

GRAM STAIN

This is the most commonly used differential stain. Candida species are said to be Gram positive and hence they retain the colour of crystal violet after being treated with iodine and alcohol and hence candida appear purple or bluish purple.

Addition of Parker's ink or the lactophenol cotton blue stain, enhances the demonstration of fungal elements. Recent development involves use of calcofluor white, a fluorochrome with an affinity for chitin and glucan which makes demonstration of fungal elements with a fluorescent microscope relatively simple.

Direct examination of skin or nail material, reveals the oval thin-walled yeasts bud on a narrow base, and are usually accompanied by filaments, either true hyphae or pseudohyphae. Presence of mycelial form shows colonisation and tissue invasion and therefore their demonstration is significant.

CULTURE

The routine medium used for isolation of fungi in culture from mucocutaneous infections is Sabouraud's Dextrose Agar (SDA) supplemented with chloramphenicol, gentamicin and / or tetracycline to

prevent bacterial overgrowth. *Candida* species from mucocutaneous sources are relatively easily cultured. Cultures can be incubated at 37°C and candida colonies will be apparent within 2-3 days. In some cases growth will be noted after 24 hours, but it may take more than 3 days.

The colonies are cream coloured, smooth, pasty, dry and wrinkled.

Sub-culture on indicator media

Chromogenic agars have now been developed that allow the identification of candida species. On Albicans ID agar (Bio Merieux), the colonies of candida albicans are blue and all other yeasts creamy or white. On chromagar (Becton & Dickinson), colonies of *C.albicans*, *C.tropicalis* and *C.krusei* are green, blue and pink respectively.

On Himedia CHROM agar various species produce the following colony colours.

Candida species		Colony colour
Candida albicans	-	Green
Candida tropicalis	-	Blue Grey or Purple
C.glabrata	-	Light Pink
C.parapsilosis	-	White, Pale Pink
C.guilliermondii	-	Pale Pink, Purple
C.krusei	-	Pink , dry
C.dubliniensis	-	Dark Green

GERM TUBE TEST

The culture of candida species is inoculated into 0.5ml of sterile human serum and incubated at 37°C for 2-4 hours. A drop of suspension is examined on a slide under the microscope. The germ tubes are seen as long tube - like projections extending from the yeast cells (hand mirror) appearance. There is no constriction at the point of attachment to the yeast cell.

Germ tubes are nothing but rudimentary true hyphae.

Demonstration of the germ tube is also known as Reynolds - Braude phenomenon. The germ tube formation commonly occurs with candida albicans. The only other species that is germ-tube positive and produces vesicles on depleted media is *C.dubliniensis*, a yeast associated predominantly, with oral infections in HIV positive patients.

CHLAMYDOSPORE FORMATION

The strain of candida isolate is grown on the corn meal agar with 1% Tween 80. It shows the formation of large, highly rounded refractile vesicles termed chlamydospores (8-12 μm diameter), at the sides and ends of the filaments. These are produced within 24-96 hours of incubation at 26°C.

BIOCHEMICAL TESTS

- The biochemical tests like sugar fermentation and assimilation are important for the identification of yeast isolates.
- Sugar fermentation is being carried out with sugars like Glucose, maltose, Sucrose and Lactose.
- Sugar Assimilation - Glucose, Sucrose, Maltose, Lactose, Galactose, Raffinose and Xylose can be used.

SEROLOGY

Mannan is a major structural component of the cell wall of the yeasts and the principal surface antigen that is available for immune interaction with colonised or infected host. It is a large molecular weight protein polysaccharide whose carbohydrate portion consists of repeating mannose units in (1,6) linkage and numerous (1,2) and (1,3). Oligomannoside side chains. The whole cell agglutination by specific antisera identifies two major serotypes of candida albicans, type A and B and mannan comprises the type specific antigen. The serological tests available currently lack specificity and sensitivity. Serum antibodies and cell-mediated immunity are demonstrable in most people as a result of life long exposure to candida. In systemic candidiasis, antibody titres to various candidal antigens may be elevated, but there are no clear criteria for establishing a diagnosis serologically.

ANIMAL PATHOGENECITY

Rabbits and mice are the most commonly used experimental animals in the mycology laboratory. They are susceptible to different species of candida.

INDICATOR MEDIA - Himedia CHROMagar

CHROM agar was founded by Dr. Alain Rambach an Internationally recognised microbiologist and pioneer in the field of Chromogenic media for the detection of pathogenic micro-organisms. With its headquarters and laboratory located in Paris near the Pasteur Institute, CHROMagar is currently developing an international sales and distribution network.

The products of Dr. Rambach's current research are "New First generation Chromogenic media and second generation (multicolor) Chromogenic media". CHROMagar Candida is a chromogenic media for the isolation, detection and differentiation of different Candida species. Yeasts produce coloured colonies within 24-48 hrs.

Over 95% of *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata* were correctly identified on the basis of colony morphology and pigmentation on CHROMagar Candida. CHROMagar Candida contains chloramphenicol to inhibit bacterial contamination. Sabouraud Dextrose Agar (SDA) is not a differential

medium, and colonies of different pathogenic yeast species that grow on this agar cannot be easily distinguished from each other. The high overall prevalence of yeast isolations in clinical laboratories has led earlier to the design of at least three isolation media intended to differentiate *Candida albicans* from other yeasts on the basis of colony colour (58).

Nickerson's medium (59) which is essentially the same as the commercially available Biggy Agar, relies on the differential reduction of complex bismuth salts to give light and dark-coloured colonies.

Pagano et al., (60) added triphenyl tetrazolium chloride as an indicator to Sabouraud agar. On this medium, *Candida albicans* isolated give pale-coloured colonies, while other yeast species develop various shades of pink. The medium has been used successfully to reveal mixed yeast species in clinical isolates.

Costa and de Lourdes branco (61) devised a phosphomolybdate agar on which *Candida albicans* colonies are green and those of other species are blue. Despite the independent studies confirming the value of these differential isolation media, none of them has achieved wide acceptance for use in the routine isolation of clinically important yeasts.

The Pagano - Levin and phosphomolybdate agar are not currently available from commercial sources, and Pagano - levin medium (62) in practice yields a high rate of both false - positive and false-negative

results when used to differentiate species. Bismuth - based media do not adequately differentiate yeast species from each other or from bacteria since most organisms form colonies with a brown to black colour on this substrate.

CHROM agar Candida medium is used for the routine isolation and presumptive differentiation of yeasts. An indicator medium should exhibit several properties. It should support the growth of yeasts but not of bacteria. The differential property of the medium should allow unambiguous presumptive discrimination between the yeast species most commonly encountered in clinical samples and it should facilitate the recognition of specimens containing mixture of yeast species and exposure of the fungi to the differential indicator substances should not affect their viabilities for subsequent subculture. CHROMagar Candida appears to fulfill all of these requirements.

The new medium supported the growth of clinically isolated yeasts and most of the moulds (with exception being chiefly among the dermatophytes) but evidently retards the growth of bacteria. The viability of fungi grown on CHROMagar candida is not affected by exposure to the medium, and the medium affords an extremely high level of discrimination among the most commonly isolated yeast species. The colour differential between *C.albicans*, *C.krusei*, *C.tropicalis* and most other species is much more striking than that seen

with previous differential yeast media (Nickerson's medium, Pango-Levin medium) with Himedia CHROMagar. The performance of CHROMagar Candida exactly paralleled that of Sabouraud glucose agar in terms of its ability to support the isolation of yeasts from clinical samples.

TREATMENT

The fungal infections these days are becoming very common not only as primary diseases but secondary to various predisposing factors. Moreover, it is very difficult to treat these infections particularly among the immunocompromised individuals. Though there have been multiple antifungal agents developed so far, only a few are clinically effective. There are many obstacles in the management of fungal diseases by these agents. Due to the eukaryotic nature of both fungal and human cells, their metabolism resembles significantly with each other. Therefore any antifungal drug inhibiting particular metabolic activity will be toxic for use by human beings also. There is poor penetration of the drug in the tissue because the fungi infect relatively poorly vascularised areas. Moreover, slow growth of fungi and the granulomatous response of host tissue also make the drug less penetrable to reach the target sites. The efficacy of the treatment of fungal infections is dependent on several factors.

1. Drug factors such as potency of the drug tissue penetration and distribution within the body.
2. Organism factors such as virulence, susceptibility to a given drug and development of resistance and
3. Host factors such as underlying immune status including the alteration of the normal mucosal flora, neutropenia and humoral and cellular immunity.

Based on the source, the antifungal agents are divided into the following groups.

1. Antifungal Antibiotics
2. Synthetic Antifungal Agents

ANTIFUNGAL ANTIBIOTICS

Polyenes

- a. Amphotericin B
- b. Nystatin

SYNTHETIC ANTIFUNGAL AGENTS

Azoles

- a. Fluconazole
- b. Itraconazole
- c. Ketoconazole

Thrush and other mucocutaneous forms of candidiasis are usually treated with topical or systemic Nystatin, Fluconazole and Ketoconazole. Systemic candidiasis is treated with Amphotericin B, sometimes in conjunction with oral flucytosine. Chronic mucocutaneous candidiasis responds well to Ketoconazole and other azoles, but patients who have a genetic defect often require life - long treatment (63 & 64).

ANTIFUNGAL AGENTS

Amphotericin B

This is a broad - spectrum polyene antibiotic which is obtained from *Streptomyces nodosus*. It has greater affinity for ergosterol than cholesterol, the latter being the predominant sterol in the mammalian cell wall. The pores formed by Amphotericin B increases permeability so that essential molecules leak from the cytoplasm and fungal growth is inhibited. It is insoluble in water and unstable at 37°C. It is a potentially effective fungicidal drug.

The intravenous route of Amphotericin B remains the gold standard of therapy for most of the fungal diseases. Amphotericin B is available in 50mg vial as deoxycholate Amphotericin B suspension. It is not absorbed after oral intake and hence, given through intravenous infusions in 5% dextrose over 2-4 hours. Intrathecal injections may also be given in case of fungal meningitis. The usual dose is 0.4 - 0.6 mg/kg/

day. The total dose should not exceed 2.0-2.5gm. The side effects of this drug restricts its uses, it is known for its nephrotoxicity. There may be headache, chills, fever, severe hemolytic anaemia, hypokalemia and nephritis.

NYSTATIN

Like Amphotericin B, Nystatin also belongs to the group of polyene antibiotic and it is obtained from *Streptomyces noursei*. It combines with the fungal cell membrane and interferes with vital cellular processes like respiration and glucose utilisation. Nystatin exhibits both fungistatic and fungicidal activity depending on the drug concentration, the susceptibility of the fungus, presence of blood.

TRIAZOLES

Fluconazole

This triazole derivative has proper water solubility, oral absorption, extensive bioavailability independent of food or pH, least protein binding and a sufficiently long half-life to allow once-a-day administration. It penetrates readily into CSF and is excreted unchanged in urine and feces being metabolically stable with recovery of over most of the administered dose.

Itraconazole

This triazole compound has been found superior to other azoles in several respects. It was first synthesized in 1980. It has better distribution in tissues and its expanded half - life is 15-24 hours. This is a lipophilic compound characterized by good absorption in all tissues except CSF.

NEW ANTIFUNGAL AGENTS

The new antifungal agents fall into several groups or drug classes. There are the triazoles, which include Posaconazole, Ravuconazole, and Voriconazole. Another class of new agents is the Candins, the beta - Glucan synthase inhibitors. Included are : (1) Caspofungin, (2) Echinocandin and (3) FK463 (micafungin); Liposomal Nystatin (Nyotran) belongs to the polyene class of antifungal drugs.

AIM AND OBJECTIVE OF THE STUDY

BACKGROUND

The term immunocompromised host is used to define a patient with impaired host defences who is at the risk of developing an opportunistic infection. This includes patients with immunodeficiency because of the disease perse such as patients with ‘Acquired Immune Deficiency Syndrome’ (AIDS) or induced iatrogenically as a result of chemotherapy. Susceptibility to infection is increased when normal host defence mechanisms are compromised by underlying disease states, therapeutic interventions or iatrogenic manipulations. Frequently all the three factors play a role in creating an immunocompromised state, in which infection is likely. Candida species are the most common cause of systemic fungal infections in the immunocompromised patients (Hawkins, 1984).

This study has been designed,

1. To isolate, culture and subculture thereby identify the subspecies of candida from the mucosal lesions of immunocompromised individuals.
2. To study the age and sex distribution of immunosuppressed patients suffering from candidiasis.

3. To study the morphological pattern of lesions in the background of immunosuppression.
4. To identify the subspecies of candida causing various types of lesions in immunosuppressed patients.
5. To study the dose and duration of the immunosuppressive therapy that predisposed to candidiasis.

MATERIALS AND METHODS

This study was conducted at the Government General Hospital, Chennai.

One hundred and Twenty (120) patients who were either on immunosuppressive therapy or suffering from acquired immunodeficiency states, attending, The Department of Dermatology were screened for the present study. Study was carried out from 1st July 2006 to 30th September 2007. Cases were included in the study after confirming their diagnosis through appropriate investigations including histopathological studies and serological studies.

INCLUSION CRITERIA

1. Patients should have cutaneous manifestations related to the disease with which they are suffering from
2. Patients should have either been instituted therapy with systemic immunosuppressive drugs for the management of their disorders or should suffer from immunodeficiency because of the disease perse.
3. Development of candidiasis should be after the institution of immunosuppressive therapy.

EXCLUSION CRITERIA

1. Patients below 12 years of age.
2. Pregnant women
3. Patients who have taken Anti-fungal treatment (topical / systemic) within the past 6 months.
4. Patients with biological false positive serological tests.

BREAK-UP DETAILS OF PATIENTS ARE AS GIVEN BELOW

Break-Up details of patients Diagnosis-Wise and Gender-Wise Screened for Candidiasis

Table - 1

Clinical Entity	Male	Female	Total
Pemphigus Vulgaris	07	18	25
Bullous Pemphigoid	08	03	11
Pemphigus Vegetans	02	03	05
Pemphigus foliaceus	03	03	06
Dermatitis Herpetiformis	02	0	02
Toxic Epidermal Necrolysis	02	01	03
Behcet's Syndrome	0	01	01
Lichen Planus	02	0	02
Systemic Lupus Erythematosus	01	03	04
Psoriasis	05	03	08
Diabetes Mellitus	02	0	02
Renal transplantation	02	0	02
HIV / AIDS	37	12	49
Total	73	47	120

Detailed case history of each patient was collected with reference to the duration of primary disease for which the immunosuppressive drug was given; appropriate note was also made regarding the dose and duration of each drug in their immunosuppressive regime.

Site, symptomatology and the duration of candidial infection that appeared after immunosuppressive therapy were recorded.

Detailed examination was also done to note down the other dermatological lesions.

MYCOLOGICAL EXAMINATION

Specimens such as mucosal scrapings were collected under aseptic precautions and examined microscopically in 10% potassium hydroxide (KOH) solution for the presence of fungal elements.

Whenever the scraping was positive for budding yeast cells, hyphae or pseudohyphae, inoculation was done on Sabouraud's Dextrose Agar (SDA) (Media of pH 6.5) with chloramphenicol (0.05mg/ml). Duplicate slants were maintained for all specimen.

All the inoculated slants were duly numbered and incubated at 37°C for a period of 24-48 hours, with everyday observation. Candida colonies appeared as white or cream coloured, smooth with a yeasty odour.

SABOURAUD'S DEXTROSE AGAR

MEDIA PREPARATION

Ingredients :

Dextrose	-	2g
Peptone	-	1g
Agar	-	2g
Distilled water	-	100 ml
pH	-	6.5

The ingredients are weighed accurately and then dissolved in 100ml of distilled water. Its then warmed gently till the boiling point of water so that agar dissolves completely and the solution becomes homogenous. When the media has become transparent warming up is stopped.

The media is sterilized by autoclaving at 15 lbs pressure (121°C) for 15 mts.

Antibiotic chloramphenicol is added (0.05mg/ml); sterilized media is then allowed to cool to 50°C and then poured into sterile test tubes and then kept in appropriate inclination for the bud and slant formation. After a time lag of 4-6 hours the media is ready for inoculation.

SUB-CULTURE MEDIA → INDICATOR MEDIA

Hicrome Candida Agar

Hicrome candida agar is recommended for rapid isolation and identification of candida species from mixed cultures.

Composition

Ingredients	Grams / Litre
Peptic Digest of animal tissue	15.0
Agar	15.0
Chloramphenicol	0.5
Chromogenic mixture	11.22
Dipotassium hydrogen phosphate	1.0
Final pH (at 25°C)	6.3 ± 0.2

Constitution of Media

21.36 gms of Hicrome candida agar is dissolved in 500 ml of distilled water. It is allowed to boil to dissolve the medium completely. Media should not be autoclaved. Media is then cooled to 50°C and aseptically poured into sterile petri plates.

Subculture

Subculture of candida from SDA in Hicrome candida agar is done after 24-48 hours of primary culture.

Principle and interpretation

Perry and Miller (65) reported that *Candida albicans* produces an enzyme β -N-acetylgalactosaminidase and according to Rousselle et al., (66) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth media helps in identification of *Candida albicans* isolates directly on primary isolation. Hicrome *Candida* agar media are selective and differential medium which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation on the basis of colouration and colony morphology.

Peptic digest of animal tissue, yeast extract, malt extract and glucose provides nitrogenous, carbonaceous, compounds and other essential growth nutrients. Chloramphenicol suppresses bacteria.

Cultural response

Organisms	Colour
<i>C.albicans</i>	- Light green
<i>C.tropicalis</i>	- Light blue
<i>C.krusei</i>	- White fuzzy
<i>C.glabrata</i>	- Light pink
<i>C.dubliniensis</i>	- Dark green

OBSERVATION AND RESULTS

Out of 120 patients with immunosuppression subjected for the screening of candidiasis, 100 patients only were positive for candidiasis which includes 62 males and 38 females (Table - 2). Their age ranged from 17 years to 85 years with a mean of 41.6 years.

Age and Gender Distribution of cases positive for candidiasis

Table - 2

Age Group (Years)	No.of Cases		Total No.of cases
	Male	Female	
10 - 20	02	00	02
21 - 30	09	13	22
31 - 40	20	10	30
41 - 50	15	08	23
51 - 60	06	04	10
61 - 70	09	02	11
71 - 80	00	01	01
81 - 90	01	00	01
Total	62	38	100

Both the patients in the extremes of age were males. The maximum number of Males (20) were in the age group of 31-40 years and maximum number of Females (13) were in the age group 21-30 years. The duration of their skin disease ranged from 3 weeks to 10 years.

Out of the 100 patients positive for candidiasis, 60 patients suffered from various dermatological entities among which pemphigus vulgaris patients top the list (24) cases. The remaining 40 patients were victims of HIV.

Pemphigus vulgaris was the commonest bullous disorder that accounted for oral candidosis (40%).

Distribution of candidiasis in relation to various dermatoses on immunosuppressive therapy is as given below.

Distribution of candidiasis in relation to various dermatoses on immunosuppressive therapy or immunocompromised status

Table - 3

Clinical Entity	Male	Female	Total
Pemphigus Vulgaris	06	18	24
Bullous Pemphigoid	08	02	10
Pemphigus Vegetans	01	02	03
Pemphigus foliaceus	03	02	05
Dermatitis Herpetiformis	01	0	01
Toxic Epidermal Necrolysis	01	0	01
Behcet's syndrome	0	01	01
Lichen Planus	02	0	02
Systemic Lupus Erythematosus	01	03	04
Psoriasis	04	01	05
Diabetes Mellitus	02	0	02
Renal transplantation	02	0	02
HIV / AIDS	31	09	40
Total	62	38	100

**Distribution of candidiasis in relation to various
dermatoses percentage wise**

Table - 4
(n = 60)

Primary Dermatoses	% of cases
Pemphigus vulgaris	40%
Bullous pemphigoid	16.6%
Pemphigus foliaceus	8.3%
Psoriasis	8.3%
Pemphigus vegetans	5%
SLE	6.6%
Lichen planus	3.3%
Diabetes mellitus	3.3%
Renal transplant	3.3%
Dermatitis herpetiformis	1.6%
Behcet's syndrome	1.6%
Toxic epidermal necrolysis	1.6%

Duration and drug regime of immunosuppressive therapy

The duration of the therapy that predisposed to candidosis ranged from 10 days to 1 year.

Out of 100 patients, positive for candidiasis 28 patients had taken treatment with Tab. Prednisolone 5mg alone taken in a dose range of 15mg to 20mg per day.

22 patients were treated with Inj. Dexamethasone (8 mg)

5 patients were on Tab. Methotrexate 7.5mg/week.

2 patients were on combination of drugs viz.,

Inj. Dexamethasone (100 mg) &

Inj. Cyclophosphamide (500 mg) (DCP) Pulse therapy.

Other combination of immunosuppressive drugs used were :

T. Prednisolone (15 mg) + Azathioprine (50 mg).

MORPHOLOGY OF THE COLONIES

After inoculation of the material under sterile aseptic precautions, in SDA culture tubes with chloramphenicol, tubes were incubated at 37°C. Creamy white, smooth individual colonies of candida with a yeasty odour were observed after 24-48 hours.

Individual colonies could be appreciated standing out as dome shaped growth till 48-72 hours, since the time of inoculation, after which the culture gets contaminated with saprophytic bacteria and other yeasts and moulds.

Subculture was made into Hicrome candida agar under sterile aseptic precautions and colonies were observed after 24-48 hours. Species of candida were identified with the help of the colour that they have produced by their interaction with the chromogenic indicator incorporated in the Himedia ChromAgar.

The following were the colours produced by difference species of candida.

Organisms	Colour
C.albicans	- Light green
C.tropicalis	- Light blue
C.krusei	- White fuzzy
C.glabrata	- Light pink
C.dubliniensis	- Dark green

Even the subculture gets contaminated after 24-48 hours after inoculation and hence meticulous care was taken to read the colours at the end of 48 hours, as the colours produced by various species gets altered because of the contaminant growth.

Germ tube formation

Species of candida albicans were inoculated into 0.5ml of sterile human serum at 37°C and incubated for 2-4 hours. When a drop of culture was examined under the high power of Microscope, candidia albicans species revealed ‘germ-tube’ formation, which is nothing but rudimentary hyphae attached to the budding yeast cell without constriction at the point of attachment, which resembled that of “Hand-mirror appearance”.

DISCUSSION

In our present study of candidiasis, out of the 100 cases positive for candidiasis included, majority of the patients were found to be in the age group of 31-50 years (53%); out of which 35 were male patients, and 18 were female patients. Patients in the extremes of age were males. The youngest male patient with oral candidiasis was a case of Bullous pemphigoid of age 17 years. The eldest patient was again a male of age 85 years, who was suffering from bullous pemphigoid.

Out of the 100 cases included in this study, 60 patients had various dermatoses and 40 cases were victims of HIV infection.

Of the 60 patients, with various dermatoses studied, pemphigus vulgaris was the commonest clinical entity to be associated with oral candidosis.

24 cases of pemphigus vulgaris had oral candidosis in our study (40%). The next common bullous disorder to be associated with candidosis was bullous pemphigoid. 10 cases of bullous pemphigoid had oral candidosis amounting to 16.6%.

Incidence of oral candidosis is more in pemphigus vulgaris than bullous pemphigoid because of the fact, that nearly 70% of the cases of pemphigus vulgaris have oral lesions, whereas only 30% of the Bullous pemphigoid cases present with oral lesions.

Other dermatoses to be associated with oral candidiasis in the decreasing order of frequency were,

Pemphigus foliaceus (8.3%), Psoriasis (8.3%), Pemphigus vegetans (5%), SLE (6.6%), Lichen planus (3.3%), Dermatitis Herpetiformis (1.6%), TEN (1.6%) and Behcet's Syndrome (1.6%).

Diabetes Mellitus was associated with candidosis to an extent of 3.3%.

2 male patients had diabetic balanoposthitis. Post renal transplant patients on immunosuppressive therapy were associated with oral candidosis to an extent of 3.3%.

IMMUNOSUPPRESSIVE DRUG

In the present study the immunosuppressive drug commonly used was prednisolone, either alone or in combination with azathioprine or cyclophosphamide. The period of immunosuppressive therapy needed to produce candidosis infection of the mucosal surfaces varies from a minimum of 10 days to maximum of 1 year. Combination of immunosuppressive drugs leads to persistence of candidial infection.

MORPHOLOGICAL TYPES OF THE LESION

In patients with bullous disorders who were on systemic steroids and other cytotoxic drugs like cyclophosphamide, azathioprine etc. the commonest type of oral candidosis encountered was **acute**

pseudomembranous candidiasis, (Thrush) characterized clinically by a sharply defined patch of creamy, crumbly, curd like white pseudomembrane, which when removed leaves an underlying erythematous base. The clinical changes were of the erosive type with severe symptoms resulting in inadequate food intake because of pain. (Photo No.1)

Acute erythematous candidosis was the second commonest type of candidosis that was encountered in our study. Marked soreness and denuded atrophic erythematous mucous membranes were noticed on the dorsal aspect of the tongue. This type might follow pseudomembranous candidiasis (Photo No.2).

Angular cheilitis (Perleche)

Soreness at the angles of the mouth extending outwards in the folds of the facial skin was noticed in all the patients of bullous dermatoses apart from their thrush lesions (Photo No.3).

Median rhomboid glossitis

Median rhomboid glossitis type of candida lesion was encountered in one HIV infected male patient of age 40 years who was on antiretroviral therapy; condition was characterized clinically by a more or less diamond shaped area on the dorsum of the tongue with loss of papillae (Photo No.4).

In cases of **chronic oral candidosis** deep fissuring of the tongue in a **cerebriform pattern** was noticed in a female patient suffering from pemphigus vulgaris (Photo No.5).

In patients with HIV infection deep longitudinal midline fissuring of the tongue was noticed (Photo No.6).

Majority of the male patients with HIV infection, with oral candidiasis had Addisonian type of oral pigmentation involving the hard and soft palates and also the buccal mucosa irrespective of their smoking habits. In HIV patients with CD₄ count less than 100 cells/Cu.mm, candidial patches were also noticed in the hard and soft palate apart from the lesions over the tongue (Photo No.7).

The black pigmentation persisted even after the clinical cure of the lesions (Photo No.8).

A female patient with HIV infection had multi-dermatomal herpes zoster as an associated dermatological entity (Photo No.9).

In Diabetic individuals candidial balanoposthitis was associated with soddening and linear fissuring; prepuce was edematous and there was subprepuccial discharge because of the associated phimosis (Photo No.10).

The following species of candida, were isolated on doing subculture in Himedia CHROMagar, viz.,

Candida albicans (52%), *C.tropicalis* (22%), *C.glabrata* (21%), *C.krusei* (3%) and *C.dubliniensis* (2%), (Fig.No.5).

Candia albicans was the **commonest** species isolated both from HIV (27) and Non-HIV (25) infected individuals.

Germ tube formation (Reynolds-Braude) phenomenon was positive in *candida albicans* on incubation with human serum at 37°C after 2-4 hours (Photo No.11). 'Fringe of pseudohyphae' formation was there around the edge of the colony in cultures of *candida albicans* (Photo No.12).

Candida dubliniensis was isolated from the oral lesions of two HIV positive patients. *C.dubliniensis* produces dark green colour in Himedia - CHROMagar (Photo No.13).

In the female patient with *C.dubliniensis* infection the CD₄ count was less than 50 cells / cu.mm and the patient had verruca vulgaris as an associated opportunistic infection. Acute pseudomembranous glossitis was the clinical type of lesion in this patient.

The commonest systemic disease associated with HIV patients with oral candidosis was pulmonary tuberculosis.

Few patients (2) also had associated tuberculous abdomen and pleural effusion.

Extensive Dermatophyte infections, Eosinophilic folliculitis, Pyrexia of Unknown Origin (PUO) and seborrhoeic dermatitis were the other associated conditions found in HIV patients.

Acneiform eruptions, skin tags, thyrotoxicosis, cortical cataract, photosensitivity, xerosis, folliculitis were the associated entities encountered in Non-HIV patients.

Candidiasis is the commonest mycosis in patients on systemic immunosuppressive therapy / immunosuppressed status (Dreizen et al., 1992) (67), Hawkins et al., 1984 (68). In symptomatic patients with candidiasis, minimum period of immunosuppressive therapy required was 10 days in a female patient with pemphigus vulgaris (age : 45 years) who was on parenteral steroid therapy, which is little earlier compared to the study by Rubin et al., in 1981.

The clinical type of candidiasis was pseudomembranous glossitis in the majority of patients as also reported in other studies (Prentice, 1989).

Disseminated candidiasis was not seen in any of the patients in this study, since dissemination is expected only with defects in the polymorphonuclear leucocyte and not with the T cell suppression alone which occurs commonly with immunosuppressive therapy.

Out of the 100 patients positive for candidosis, diabetes mellitus was found in 16 patients; of these sixteen patients (6 patients were females and 10 were males), Diabetes mellitus was exclusively present in only 2 male patients as a predisposing factor for candidosis (Balanoposthitis) whereas in the remaining 14 patients primary dermatoses like bullous disorders and immunosuppression due to HIV infection were the main predisposing factors for candidial infection, and diabetes mellitus was just an added factor for predisposition.

Highest incidence of 'O'+ve blood group was noticed in majority of the patients with candidiasis in our study; this was followed by B+ve which is similar to the occurrence in general population, although people with O+ve Blood group are considered to have high carriage of candida species.

SUMMARY AND CONCLUSIONS

- **Candidiasis** is the **commonest** mycosis in patients on systemic immunosuppressive therapy and immunosuppressive status like HIV infection.
- Majority of the affected victims of candidiasis, were in the age group between 31-50 years. Incidence of candidiasis was found to be more in male patients than female patients. (Males → 62%). (Females → 38%). However the age and sex distribution is also related to the patients screened.
- **Acute pseudomembranous glossitis** was the commonest morphological type of the lesion encountered in this study (35%), to be followed by, chronic erythematous candidiasis (22%), Angular cheilitis (21%), acute erythematous glossitis (19%), balanoposthitis (2%) & Median rhomboid glossitis (1%).
- Among the various immunosuppressive drugs taken into account for this study, **Tab.Prednisolone** was the commonest drug predisposing to candidiasis (28%) followed by Inj. Dexamethasone (22%). Thus systemic steroids accounted for 50% of drugs predisposing to candidosis, remaining 50% being contributed by cytotoxic drugs like methotrexate, cyclophosphamide, azathioprine etc;

- Of all the various bullous dermatoses screened, pemphigus vulgaris was the commonest bullous disorder predisposing to candidosis (40%) followed by bullous pemphigoid (16.6%), Pemphigus foliaceus (8.3%), Dermatitis Herpetiformis (1.6%) and Pemphigus vegetans (5%).
- **Candida albicans** was the commonest species isolated (52%). Other species isolated include, Candida tropicalis (22%), Candida glabrata (21%), Candida krusei (3%) and Candida dubliniensis (2%).
- Patients with O+ve Blood group are considered to have high carriage of candidia species.
- Even in **HIV infected individuals**, **candida albicans** was the commonest species isolated, in our study; **candida dubliniensis** is said to be exclusively common in the oral lesions of HIV patients, which however accounted only for (2%) in our study. Probably the sample size had still been larger.
- The minimum time duration of systemic immunosuppressive therapy required for predisposition to oral candidosis was found to be 10 (Ten) days in our study in Non-HIV infected individuals,

which was found to be still shorter in known cases of HIV infection (around 1 week); probably due to CD₄ cell count depletion, and other associated opportunistic infections.

- In spite of heroic measures to avoid contaminants, and adhering to very strict aseptic protocols, it was found in our study that the maximum keeping time of primary candida culture in SDA with chloramphenicol and subcultures in Himedia CHROMagar was just 72 hours, after which saprophytic bacteria and various moulds and concomitant pathogens spoil the culture media.

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PROFORMA

Sl.No.

Name :

O.P.No. :

Age :

Sex : M/F

Date :

Chief Complacints :

Duration :

Family History :

Marital History :

Predisposing Factors :

Diabetes

Tuberculosis

Immunosuppressive Therapy

HIV

Pregnancy

Malnutrition

Endocrine Causes

Systemic Illness

Iatrogenic Causes

Associated dermatological disorders :

Acne

Sebderm

Psoriasis

Lichen Planus

Dermatophyte Infection

Acanthosis Nigricans

Other Dermatological Conditions

General Examination :

Anemia

Obesity	Weight
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100	100

Avitaminosis

Diet - Nutritional Status

Pregnancy

TB

Systemic Examination

CVS

RS

Abdomen

CNS

Others

Dermatological Examination

Site of the lesion

Type of the lesion

Cutaneous (or) Mucocutaneous

INVESTIGATIONS

1. Routine Haemogram
2. Scraping / Swab (KOH examination)
3. Special stains
4. Culture and Subculture
5. Blood grouping & Typing
6. Blood VDRL
7. HIV ELISA
8. Blood Sugar
9. Endocrine Assessment